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Application No. 09/869,638

Filed: February 8, 2002

TC Art Unit: 2624

Confirmation No.: 5891

AMENDMENT TO THE CLAIMS

1. (Currently Amended) A method for the automatic analysis of microscope images of biological objects, in particular for the analysis of fluorescence images of cells, comprising the following steps:

- a) taking at least two microscope images of a sample including a plurality of biological objects;
- b) selecting a first microscope image and marking the position(s) of mass gravity centers of a number n of the individual objects discernible in the first microscope image, in which step each marked object is assigned a defined first image excerpt which completely surrounds the marked object, and each first image excerpt including a marked object is assigned the value 1, with the number n of such marked first image excerpts constituting a positive training set;
- c) selecting and marking a number m of second image excerpts in said first microscope image each spaced a predetermined minimum distance from said first image excerpts, with a second image excerpt corresponding in size and shape to said first image excerpt, in which step each second image excerpt is assigned the value 0, with the number m of such marked second image excerpts constituting a negative training set;
- d) determine characteristic features and/or feature combinations of the positive and negative training sets and assigning said characteristic features and/or feature combinations to a classification value between 0 and 1, said classification value representing the degree of probability

Application No. 09/869,638

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of the presence of a marked object, and the determined features and/or feature combinations are stored;

- e) determine classification values of all image points of the second and each further microscope image by comparing the image data of the second and each further microscope image with the features and/or feature combinations in said first microscope image determined in procedural step d), in which step, for each image point of the second and each further microscope image, the classification value for an image excerpt surrounding the image point is determined and the size and shape of this image excerpt corresponds to the size and shape of the first or second image excerpt; and
- f) recognizing the position(s) of biological objects in the second or each further microscope image by evaluating the determined classification values, in which step the determined classification values are compared with a given threshold value representing the presence of a biological object, wherein classification values of all image points of the second and each further microscope image are automatically determined according to procedural step e) by scanning the image surface of the second and each further microscope image and wherein, further, the object positions determined by procedural steps a) to f) are compared in the total number of microscope images so as to obtain a spatial location and distribution of the individual objects in the sample; and wherein the biological objects to be determined are marked with a different set of one or plural chemical markers before each microscope image is taken, and a bleaching or rinsing procedure is performed between taking

Application No. 09/869,638

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TC Art Unit: 2624

Confirmation No.: 5891

an image and taking the next image; and wherein at least one
said bleaching procedure is performed.

2. (Original) The method as claimed in claim 1 wherein the sample is a tissue sample and the biological object is a cell.

3. Canceled.

4. Canceled.

5. (Previously Presented) The method as claimed in claim 1 wherein said chemical markers are fluorochrome markers and the microscope images are fluorescence images.

6. (Original) The method as claimed in claim 1 wherein the microscope images are taken by a CCD camera and then digitized.

7. (Original) The method as claimed in claim 1 wherein the number n of the individual biological objects marked in procedural step b) is larger than or equal to 50.

8. (Original) The method as claimed in claim 1 wherein the first image excerpt is of square shape, with the size and/or side length of the first image excerpt corresponding at least to the maximum diameter of the biological objects in the first microscope image.

9. (Previously Presented) The method as claimed in claim 1 wherein the number m of second image excerpts is larger than or equal to 50, with the second image excerpts being defined automatically,

Application No. 09/869,638

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TC Art Unit: 2624

Confirmation No.: 5891

keeping to the minimum distance from the respective first image excerpts.

10. Canceled.

11. (Original) The method as claimed in claim 1 wherein the threshold value of the classification value representing the presence of a biological object is at least 0.5.

12. Canceled.

13. (Previously Presented) A method as claimed in claim 1 wherein fluorescent cells in said sample are automatically classified.

14. Canceled.

15. (Previously Presented) The method as claimed in claim 1 wherein said chemical markers are fluorochrome markers and the microscope images are fluorescence images.